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REVIEW

RAGE: a new frontier in chronic airways disease

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Asthma and chronic obstructive pulmonary disease (COPD) are heterogeneous inflammatory disorders of the respiratory tract characterized by airflow obstruction. It is now clear that the environmental factors that drive airway pathology in asthma and COPD, including allergens, viruses, ozone and cigarette smoke, activate innate immune receptors known as pattern-recognition receptors, either directly or indirectly by causing the release of endogenous ligands. Thus, there is now intense research activity focused around understanding the mechanisms by which pattern-recognition receptors sustain the airway inflammatory response, and how these mechanisms might be targeted therapeutically. One pattern-recognition receptor that has recently come to attention in chronic airways disease is the receptor for advanced glycation end products (RAGE). RAGE is a member of the immunoglobulin superfamily of cell surface receptors that recognizes pathogen- and host-derived endogenous ligands to initiate the immune response to tissue injury, infection and inflammation. Although the role of RAGE in lung physiology and pathophysiology is not well understood, recent genome-wide association studies have linked RAGE gene polymorphisms with airflow obstruction. In addition, accumulating data from animal and clinical investigations reveal increased expression of RAGE and its ligands, together with reduced expression of soluble RAGE, an endogenous inhibitor of RAGE signalling, in chronic airways disease. In this review, we discuss recent studies of the ligand–RAGE axis in asthma and COPD, highlight important areas for future research and discuss how this axis might potentially be harnessed for therapeutic benefit in these conditions.

Abbreviations

COPD, chronic obstructive pulmonary disease; DAMPs, damage associated molecular patterns; HMGB1, high-mobility group box-1; PAMPs, pathogen associated molecular patterns; RAGE, receptor for advanced glycation end products; SAA, serum amyloid A; TLR, toll-like receptor

Introduction

Asthma and chronic obstructive pulmonary disease (COPD) are heterogeneous inflammatory disorders of the respiratory tract characterised by airflow obstruction, which is usually variable and reversible in asthma, but largely fixed or irreversible in COPD. The hallmark of both conditions is the development of an exaggerated chronic inflammatory response in the lungs, resulting from an abnormal immune response to diverse environmental exposures, including pollutants and

particulate matter, inhaled allergens and respiratory pathogens. Cigarette smoking is the most important risk factor for the development of COPD, while respiratory viral infection and sensitization to inhaled allergens in early life are strongly implicated in asthma pathogenesis (Anderson, 2008; Barnes, 2008b; Brusselle *et al.*, 2011).

Asthma and COPD are associated with distinct patterns of airway inflammation, which in turn lead to distinct structural abnormalities of the airways and lung parenchyma in each condition. It is now clear, however, that activation of *innate immune pathways* in response to the inhaled inciting agent(s) in genetically susceptible individuals is the basic premise of disease pathogenesis in both asthma and COPD (Lambrecht and Hammad, 2010; Brusselle *et al.*, 2011). The innate immune system forms the first line of host defence against the inhaled environment of the lung. Innate immune cells lining the airway mucosal surface sense potential threats or 'dangers' through the expression of innate immune receptors known as *pattern-recognition receptors*. Pattern-recognition receptors consist of a number of receptor families, each of which play a role in the recognition of pathogen-associated molecular patterns (PAMPs). However, it is now clear that the environmental factors that drive airway pathology in asthma and COPD can also activate pattern-recognition receptors indirectly by causing tissue damage and the release of endogenous molecules, otherwise known as damage-associated molecular patterns (DAMPs) (Chen and Nuñez, 2010b). Upon recognition of pathogen- or damage-associated molecular motifs, pattern-recognition receptors elicit the activation of innate immune responses, which then engage adaptive immune responses, as appropriate, to eliminate the source of danger and restore tissue homeostasis. As such, there is now intense research activity focused around understanding the mechanisms by which pattern-recognition receptors sustain the airway inflammatory response and how these mechanisms might be targeted therapeutically.

One of the few pattern-recognition receptors that can recognize both PAMPs and DAMPs is the receptor for advanced glycation end products (RAGE). Implicated in the pathogenesis of numerous chronic conditions including cardiovascular, metabolic and neurodegenerative disorders, RAGE contributes to both inflammatory and tissue remodelling processes (Salminen *et al.*, 2009; Yan *et al.*, 2010). Ironically, while RAGE was first isolated from lung tissue 20 years ago and is most highly expressed in the lung compared with all other organ tissues, its role in lung physiology and pathophysiology is not well understood (Mukherjee *et al.*, 2008; Buckley and Ehrhardt, 2010). Nevertheless, the emerging evidence points towards a fundamental pathogenic role for RAGE in chronic airways disease. In this review, we provide an update of RAGE biology, discuss recent studies of the ligand-RAGE axis in asthma and COPD, highlight important areas for future research and discuss how this axis might potentially be harnessed for therapeutic benefit in these conditions.

RAGE ligand recognition and cellular signalling

RAGE is a member of the immunoglobulin superfamily of cell surface receptors. It was first discovered in 1992 as a binding receptor for advanced glycation end products (AGEs), a heterogeneous group of non-enzymatically glycosylated proteins or lipids that accumulate as a result of normal aging or inflammatory processes, particularly in diabetes (Neeper *et al.*, 1992; Schmidt *et al.*, 1992; Yan *et al.*, 2010). Over the past 15 years, however, RAGE has emerged as a key pattern-recognition receptor capable of binding a diverse repertoire of soluble and cell-associated molecules involved in the host response to tissue injury, infection and inflammation, thus generating much interest in its role in disease. RAGE ligands identified to date include high-mobility group box-1 (HMGB1) (Hori *et al.*, 1995), serum amyloid A (Yan *et al.*, 2000; Cai *et al.*, 2007), amyloid β peptide (Yan *et al.*, 1996), complement 3a (C3a), heat shock protein 70 (HSP70), the matricellular injury-related glycoprotein SPARC (secreted protein acidic and rich in cysteine) (Ruan *et al.*, 2010), several members of the S100 protein family (Leclerc *et al.*, 2009), the β2-integrin Mac-1 (CD11b) (Chavakis *et al.*, 2003; Orlova *et al.*, 2007; Frommhold *et al.*, 2010) and phosphatidylserine (Friggeri *et al.*, 2011; He *et al.*, 2011). Ligation of RAGE induces the activation of multiple signalling pathways that may vary depending on the ligand, cell and tissue microenvironment, and thus mediates diverse cellular responses. Individually detailing the specific signalling pathways activated down-stream of RAGE for its numerous ligands is beyond the scope of this review. However, the pathways that can be activated include NADPH oxidase, Ras, Src kinase, Ras-ERK1/2, stress-activated protein kinase (SAPK)/JNK and p38 MAPK pathways, PI3K/Akt, small GTPase Cdc42/Rac, RhoA-associated kinase (ROCK), PKC βII and glycogen-synthase kinase 3β (GSK-3β), resulting in the activation of a number of transcription factors including NF-κB, activator protein 1 (AP-1), cAMP response element binding (CREB) protein, signal transducers and activators of transcription 3 (STAT3) and early growth response-1 (egr-1) (Huttunen *et al.*, 2002; Reddy *et al.*, 2006; Shang *et al.*, 2010; Xu *et al.*, 2010; Bianchi *et al.*, 2011; Kamioka *et al.*, 2011) (Figure 1).

RAGE is a type I transmembrane receptor composed of three immunoglobulin domains located in the extracellular space, a single membrane spanning domain and a short cytoplasmic domain essential for signal transduction. The mechanisms by which RAGE interacts with such a diverse ligand repertoire and the mechanisms by which the different ligands induce distinct cellular responses have recently begun to emerge, but knowledge in this area is still very limited (Fritz, 2011). The extracellular immunoglobulin domains consist of one variable like V-domain and two constant like C type domains (C1 and C2) (Neeper *et al.*, 1992). The C2 domain is structurally independent of the V and C1 domains, which together form an integrated structural unit (VC1) important for ligand recognition (Dattilo *et al.*, 2007; Leclerc *et al.*, 2007; Ostendorp *et al.*, 2007; Xie *et al.*, 2008). Structural and biochemical studies have deduced that RAGE preassembles in the plasma membrane in the absence of ligands, and that ligand binding enhances and stabilizes RAGE dimerization or oligomerization, which is essential for initiation of RAGE signal transduction (Ostendorp *et al.*, 2007; Xie *et al.*, 2008; Koch *et al.*, 2010; Zong *et al.*, 2010). Because RAGE ligands have a tendency to oligomerize, it is thought that the level of RAGE clustering induced by bound ligands, as well as the spatial arrangement of RAGE within these clusters might

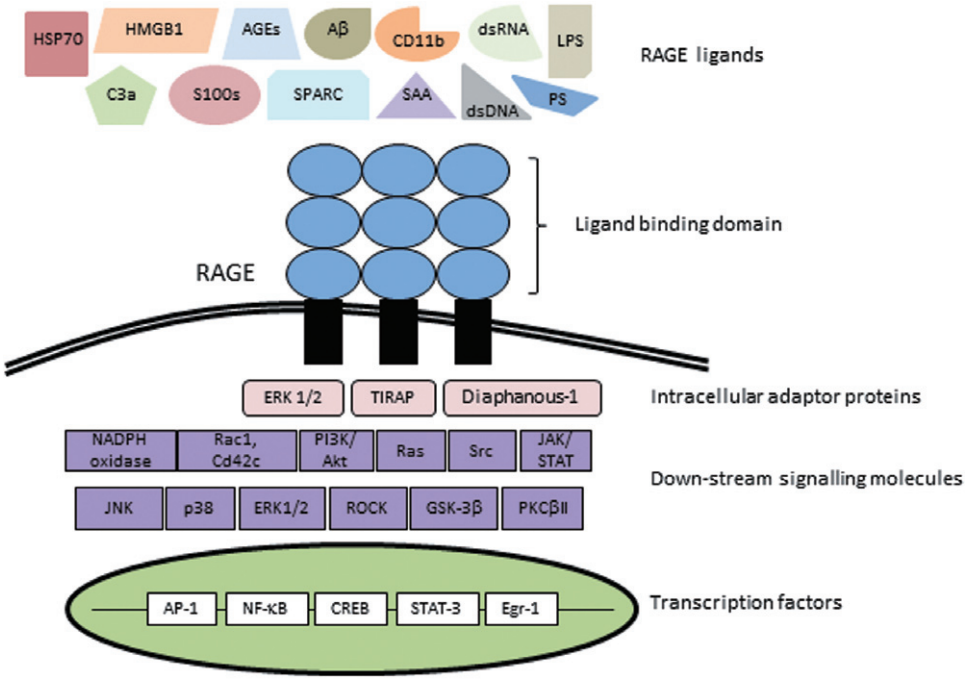

Figure 1

Schematic depicting RAGE, RAGE ligands and down-stream signalling molecules. RAGE is a pattern-recognition receptor involved in mediating cellular responses to soluble and cell-associated molecules involved in the host response to tissue injury, infection and inflammation. RAGE ligands identified to date include HMGB1, SAA, Aβ, C3a, HSP70, the matricellular injury-related glycoprotein SPARC, several members of the S100 protein family, the β2-integrin Mac-1 (CD11b), phosphatidylserine (PS), double-stranded DNA (dsDNA), double stranded RNA (dsRNA) and LPS. The RAGE cytoplasmic domain interacts with a number of intracellular adaptor proteins including diaphanous-1, ERK1/2 and TIRAP. Ligation of RAGE induces the activation of multiple signalling pathways that may vary depending on the ligand, cell and tissue microenvironment. Signalling molecules activated down-stream of RAGE include NADPH oxidase, Ras, Src kinase, Ras- ERK1/2, SAPK/JNK and p38 MAPK pathways, PI3K/Akt, small GTPase Cdc42/Rac, ROCK, PKC βII and GSK-3β, resulting in the activation of a number of transcription factors including NF-κB, AP-1, CREB protein, STAT3 and egr-1.

determine the nature and intensity of RAGE signalling in response to different ligands. This concept is supported by studies that show that the differential effects of the S100 proteins S100B and S100A6 on cell survival can be attributed to distinct interactions with the VC1, and the C1/C2 domains respectively (Leclerc *et al.*, 2007). High resolution of the crystal structure of the VC1 domain revealed large hydrophobic and positively charged regions on the surface of the V domain, which contribute to at least two distinct mechanisms of RAGE ligand recognition: in the case of S100B, calcium-dependent hydrophobic interactions are involved, while in the case of AGEs, recognition occurs primarily through binding negatively charged regions of AGE-modified proteins, rather than interaction with distinct glycation moieties on the amino acid chains of proteins (Park *et al.*, 2010; Fritz, 2011). Recent studies have also provided evidence that RAGE forms complexes with cell surface molecules, such as heparin sulphate and membrane-type 1 (MT1)-MMP), which are required for signalling by certain ligands, namely HMGB1 and AGEs respectively (Kamioka *et al.*, 2011; Xu *et al.*, 2011).

Surprisingly, very little is known about how the RAGE cytoplasmic domain engages and stimulates downstream signalling cascades (Fritz, 2011). Hudson *et al.* (2008) identified diaphanous-1 (Dai-1) as an intracellular adaptor protein that associates with the cytoplasmic domain to mediate the activation of Rac-1/CDc42 and associated cellular migratory responses. ERK-1/2 also interacts with the cytoplasmic domain, but the functional significance of this has not been determined (Ishihara *et al.*, 2003). Most recently, TIRAP and MyD88, intracellular adaptor proteins used by members of the toll-like receptor (TLR) family of pattern-recognition receptors, have also been reported to interact with the cytoplasmic domain to activate down-stream signalling cascades. PKCζ-dependent phosphorylation of the receptor was required for ligand-dependent recruitment of TIRAP and MyD88 (Sakaguchi *et al.*, 2011).

RAGE provides a molecular platform for the recognition of ligand complexes and amplification of the immune response

It is increasingly apparent that endogenously produced ligands of RAGE have important immune adjuvant properties. A notable example is HMGB1. HMGB1 forms molecular complexes with pathogen-associated molecular patterns (e.g. LPS, bacterial DNA), as well as cytokines and chemokines, and these complexes are far more powerful at stimulating immune and inflammatory responses than either HMGB1 or the partner molecule alone (Castiglioni *et al.*, 2011). Emerging evidence is consistent with RAGE providing a molecular platform for the recognition of these ligand complexes and

thus is likely to serve a central role in the amplification of immune and inflammatory responses. For example, compared with either bacterial DNA (CpG A DNA) or HMGB1 alone, molecular complexes consisting of CpG A DNA and HMGB1 synergistically enhance IFN-α release in mouse plasmacytoid dendritic cells (pDCs). Importantly, while IFN-α release induced by CpG A DNA alone is mediated by its cognate receptor TLR9, augmentation of IFN-α release induced by CpG A DNA–HMGB1 complexes involves collaborative TLR9–RAGE signalling. The CpG A DNA–HMGB1 complex induces internalisation of RAGE, and its physical association with TLR9 and its signalling adaptor protein MyD88 (Tian *et al.*, 2007). Augmentation of cytokine production induced by HMGB1 complexed with either LPS, or the inflammatory cytokine IL-1β, is also due to co-ligation of RAGE and TLR4 (the cognate receptor for LPS) or the IL-1β receptor, respectively; but whether there is functional collaboration between RAGE and TLR4 or IL-1R remains to be established (Qin *et al.*, 2009). The fact that several RAGE ligands including HMGB1 signal via TLRs (Yang *et al.*, 2010), together with accumulating evidence that (i) RAGE directly binds classical TLR ligands such as LPS and nucleic acids (Park *et al.*, 2010; Yamamoto *et al.*, 2011) and (ii) engages the TLR intracellular adaptor proteins TIRAP and MyD88 (Sakaguchi *et al.*, 2011), provides a compelling basis for molecular collaboration or synergy between RAGE and TLR ligand recognition and signalling. The elucidation and characterization of RAGE/TLR collaboration is an important next step towards deeper understanding of the host immune response and the role of these receptors in disease pathogenesis, as will be discussed in the sections below.

Soluble RAGE – an endogenous regulator of RAGE signalling

RAGE also exists as a soluble protein which is generated by two distinct mechanisms. The human RAGE gene consists of 11 exons that can be alternatively spliced to produce up to 19 mRNA splice variants (RAGE_v1 through to RAGE_v19) that lead to protein changes in the ligand-binding domain, or removal of the transmembrane or cytosolic domains of the full-length receptor (Hudson *et al.*, 2007). RAGE_v1 (esRAGE), is a secreted soluble protein which consists of the extracellular immunoglobulin domain but lacks the transmembrane and cytosolic domains. Ectodomain shedding of the extracellular domain by metalloproteinases such as MMP3, MMP9, MMP13 and ADAM10 also generates a soluble form of the receptor, commonly termed cleaved RAGE (Raucci *et al.*, 2008; Zhang *et al.*, 2008b; Yamakawa *et al.*, 2011). Secreted and cleaved forms of soluble RAGE bind ligands with similar affinity to the membrane-associated receptor, and thus are believed to function as receptor decoys by limiting the concentration of available ligands. However, alternative mechanisms are now proposed, as soluble RAGE directly binds to the membrane receptor to prevent ligand-induced homodimerisation which is essential for initiation of down-stream signalling (Koch *et al.*, 2010; Zong *et al.*, 2010; Fritz, 2011).

Despite its well documented role as an inhibitor of RAGE signalling, there is evidence that soluble RAGE has agonist functions. For example, it promotes the migration, differentiation and activation of human monocytes *in vitro* and is also chemotactic for human neutrophils (Pullerits *et al.*, 2006; Wang *et al.*, 2010). The mechanism(s) by which soluble RAGE mediates these effects is yet to be determined but appears, at least in part, to involve ligation of yet to be identified cell surface receptor(s). Of note, while soluble RAGE induces cytokine production in splenocytes via ligation of the cell surface integrin CD11b (Pullerits *et al.*, 2006), CD11b was excluded as the binding receptor responsible for monocyte differentiation, indicating that multiple and/or distinct mechanisms might be involved depending on the cell type.

Importantly, it should be mentioned that the agonist effects of soluble RAGE on monocyte and neutrophil responses mentioned above were observed under experimental conditions where RAGE ligands were not added to the system. This is significant, because in the presence of RAGE ligand, soluble RAGE has antagonist effects on monocyte responses, as evidenced by its ability to inhibit HMGB1-induced monocyte adhesion and transendothelial migration *in vitro* (Rouhiainen *et al.*, 2004). Similarly, while soluble RAGE induces cytokine release in splenocytes, it inhibits HMGB1-induced cytokine release in these same cells (Pullerits *et al.*, 2006). Thus, it is possible that under physiological conditions, where the concentration of RAGE ligands is likely to be low, soluble RAGE primarily functions as an agonist to maintain cellular homeostasis, whereas under pathological conditions where the concentration of RAGE ligands is high, soluble RAGE primarily functions as an inhibitor of RAGE signalling to promote resolution of immune and inflammatory responses. This appears to be supported by *in vivo* studies, as intratracheal administration of soluble RAGE induces neutrophil and monocyte infiltration into the lung tissue in mice, consistent with *in vitro* studies demonstrating its chemotactic activity for these cells (Pullerits *et al.*, 2006; Wang *et al.*, 2010). In contrast, when soluble RAGE is administered to mice following challenge with an inflammatory stimulus such as LPS, significant attenuation of lung neutrophilic inflammation is observed (Zhang *et al.*, 2008a).

Soluble RAGE in health and disease

Cross-sectional studies in population samples have shown inverse associations between circulating plasma concentrations of total soluble RAGE (i.e. cleaved and secreted forms) and anthropometric and physiological parameters such as body mass index (BMI) (Yamagishi *et al.*, 2006; Kim *et al.*, 2009; Norata *et al.*, 2009), C-reactive protein (CRP), glycosylated haemoglobin (HbA1c) (Basta *et al.*, 2006) and renal function (Kalousová *et al.*, 2006; Semba *et al.*, 2009); but the physiological significance of these associations is yet to be established. Importantly, however, in cross-sectional studies conducted in various chronic conditions including metabolic, cardiovascular, neurodegenerative and inflammatory disorders, as well as cancer, inverse associations between circulating concentrations of total soluble RAGE, and surrogate markers of disease risk or burden, has led to the view that soluble RAGE is a protective factor (Vazzana *et al.*, 2009). However, this has not been a consistent finding, with some studies reporting positive correlations between circulating levels of total soluble RAGE and disease risk or burden. In two prospective studies in large cohorts of subjects with type 1 diabetes, circulating total soluble RAGE concentrations were

positively associated with cardiovascular and all-cause mortality (Nin *et al.*, 2010; Thomas *et al.*, 2011).

The mechanisms underlying altered levels of soluble RAGE in the context of disease, although the subject of much debate and controversy, remain essentially unknown. Interestingly, however, a single nucleotide polymorphism (SNP) within the RAGE gene is associated with lower levels of total plasma soluble RAGE in both health and disease (Jang *et al.*, 2007; Gaens *et al.*, 2009; Kim *et al.*, 2009; Peng *et al.*, 2009; Li *et al.*, 2011b). The minor allele (T allele or CT/TT genotype) of this SNP converts glycine-to-serine at position 82 (G82S) within the RAGE ligand binding domain Moreover, this polymorphism also confers greater affinity for ligands and enhanced functional activation of the receptor (Hoffmann *et al.*, 2002; Osawa *et al.*, 2007; Park *et al.*, 2011) and is associated with greater disease risk and/or burden in healthy subjects and in chronic disease (Hofmann *et al.*, 2002; Balasubbu *et al.*, 2010; Daborg *et al.*, 2010; Gao *et al.*, 2010; Li *et al.*, 2010; 2011b). Thus, it is conceivable that individuals with this polymorphism might be susceptible to heightened RAGE-dependent inflammatory responses, which are further exacerbated by reduced expression of soluble RAGE.

The emerging role of the ligand-RAGE axis in chronic airways disease

Evidence is accumulating from clinical studies that expression of RAGE and RAGE ligands is increased in COPD subjects (Merkel *et al.*, 2005; Morbini *et al.*, 2006; Bozinovski *et al.*, 2008; 2012; Hacker *et al.*, 2009; Shang *et al.*, 2009; Ferhani *et al.*, 2010; Smith *et al.*, 2010; Kanazawa *et al.*, 2012; Hou *et al.*, 2011a,b). Ferhani and colleagues demonstrated that, compared with never smokers and smokers without COPD, smokers with COPD have increased RAGE and HMGB1 expression in the submucosa, epithelium and smooth layers of the bronchial wall and in alveolar macrophages. RAGE and HMGB1 co-localization was demonstrated in these cells and tissues, consistent with increased functional activation of RAGE signalling. Moreover, HMGB1 expression was significantly and inversely associated with airflow limitation (as indicated by FEV_1 and the diffusing capacity of the lung for carbon monoxide), indicating that increased HMGB1-RAGE signalling potentially contributes to lung function impairment (Ferhani *et al.*, 2010).

Unfortunately, Ferhani and colleagues did not determine whether increased RAGE and HMGB1 expression in the bronchial walls of their study subjects was related to changes in lung or systemic levels of soluble RAGE. However, other studies provide consistent evidence of reduced systemic levels of total soluble RAGE in COPD. In one study, systemic concentrations of soluble RAGE were reduced in stable COPD, compared with healthy subjects, and further reduced during acute COPD exacerbation, compared with convalescence (Smith *et al.*, 2010). Miniati *et al.* (2011) confirmed these observations in a larger cohort of COPD subjects and also demonstrated a positive correlation between the reduction in soluble RAGE and lung function impairment. Furthermore, they showed that COPD subjects with severe emphysema or chronic cor pulmonale have significantly lower levels of systemic soluble RAGE compared with those without. Of note, RAGE has been identified as a differentiation factor for type I alveolar epithelial (AT1) cells, both during normal development and upon regeneration from type II alveolar-epithelial (ATII) progenitor cells during alveolar injury (Dahlin *et al.*, 2004; Shirasawa *et al.*, 2004; Demling *et al.*, 2006; Reynolds *et al.*, 2008). Thus, it is possible that reduced systemic levels of soluble RAGE in emphysema could reflect the extensive disruption of alveoli and alveolar walls (Miniati *et al.*, 2011). Finally, certain RAGE ligands such as serum amyloid A (SAA) are elevated in the context of reduced systemic soluble RAGE in stable COPD and during acute COPD exacerbation, although other ligands such as S100A12 and the AGE product N-ε-carboxymethyl lysine are not (Smith *et al.*, 2010; Miniati *et al.*, 2011). Taken together, the studies to date indicate that RAGE and its ligands are elevated in COPD subjects, while total soluble RAGE is down-regulated, but whether these observations are causally related remains an open question. Indeed, prospective studies in COPD subjects are required to establish whether variation in systemic levels of soluble RAGE over time relates to disease progression, or perhaps even the development of specific components of disease, such as emphysema.

In comparison with COPD, less is known about the ligand–RAGE axis in asthma pathogenesis. There is evidence of increased expression of RAGE ligands in the airways in asthma (Vignola *et al.*, 1995; Büyüköztürk *et al.*, 2004; Ozseker *et al.*, 2006; Yang *et al.*, 2007b; Gharib *et al.*, 2011; Hou *et al.*, 2011a,b; Watanabe *et al.*, 2011); but whether there is increased RAGE expression or activity in asthmatic airways is not yet known. Hou and colleagues compared expression of the RAGE ligand HMGB1 in asthmatic and COPD subjects. While sputum and plasma HMGB1 levels were significantly increased in both asthmatic and COPD subjects compared with healthy control subjects; after adjusting for sex, age, smoking status, daily dose of inhaled corticosteroids and disease severity, HMGB1 expression was significantly greater in COPD versus asthmatic subjects. However, in both asthmatic and COPD subjects, HMGB1 levels in plasma and sputum increased with the degree of disease severity and were negatively correlated with the degree of lung function impairment (Hou *et al.*, 2011b). Thus, while there may be quantitative differences in expression and activity, it is highly likely that the ligand–RAGE pathway will emerge as an important pathogenic factor in both asthma and COPD.

Of note, two independent genome-wide association studies in healthy individuals of European ancestry reported a significant association between the RAGE G82S SNP (rs2070600) and spirometric measures of airflow obstruction (FEV_1/FVC) (Hancock *et al.*, 2010; Repapi *et al.*, 2010). As mentioned above, this SNP is associated with reduced systemic levels of soluble RAGE and enhanced functional activation of RAGE signalling. Surprisingly, however, this SNP was not significantly associated with asthma susceptibility in a genome wide association study performed in a Japanese population, although another SNP (rs404860) located in close proximity was (Hirota *et al.*, 2011). Furthermore, combined analysis of four separate COPD case-control studies involving individuals of European ancestry found this SNP was associated with protection rather than susceptibility to COPD (Castaldi *et al.*, 2011). Moreover, Young *et al.* (2011) observed increased frequency of this SNP in smokers *without* COPD, compared with those *with* COPD, suggesting that it confers

'resistance' to the development of COPD in those who smoke. Thus, while it seems clear that gene polymorphisms in and around the RAGE gene are related to variation in lung function, further studies are required to firmly establish the relationship between RAGE gene polymorphisms, environmental exposures and susceptibility to asthma and COPD across different populations.

The ligand–RAGE axis and neutrophilic inflammation in chronic airways disease

Neutrophilic inflammation is an important component of the airway inflammatory response in COPD and some phenotypes of asthma, where it is associated with more severe and treatment refractory disease (Simpson *et al.*, 2009). We observed near complete deficiency of bronchial lavage levels of total soluble RAGE in asthmatic and COPD subjects with high levels of airway neutrophils (i.e. >65% total airway cells), compared with those without and healthy control subjects. The concentration of total systemic soluble RAGE was also significantly reduced in asthmatic and COPD subjects with airway neutrophilia, compared with those without. Moreover, we found neutrophilic inflammation to be an independent predictor of total lung soluble RAGE. On the other hand, we showed that in addition to older age, lower lung function and the presence of airway bacterial colonisation, total lung soluble RAGE was an independent predictor of neutrophilic airway inflammation (Sukkar *et al.*, 2011). Of note, in contrast to other studies where increased airway expression of the RAGE ligands HMGB1 and SAA in asthmatic and/or COPD subjects was correlated with the degree of airway neutrophilia (Ozseker *et al.*, 2006; Hou *et al.*, 2011b; Watanabe *et al.*, 2011; Bozinovski *et al.*, 2012), we did not observe differences in bronchial lavage levels of HMGB1 in asthmatic and COPD subjects, irrespective of the presence or absence of airway neutrophilia, compared with healthy control subjects. However, it is possible inhaled corticosteroid use by our study subjects might have influenced this, as we observed differential HMGB1 expression when subjects were divided on the basis of corticosteroid use.

Although we observed near complete absence of lung soluble RAGE in neutrophilic asthma/COPD, systemic levels were significantly, but only modestly reduced in neutrophilic versus non-neutrophilic subjects. These observations are consistent with other studies that have sampled soluble RAGE at the site of disease versus systemically in the same subjects. Indeed, in cystic fibrosis, total soluble RAGE is completely absent in sputum samples, but readily detectable in peripheral blood (Makam *et al.*, 2009). Similarly, in rheumatoid arthritis, the levels of total soluble RAGE in synovial fluid are markedly lower than those in peripheral blood (Pullerits *et al.*, 2005). Thus, the question arises as to whether soluble RAGE deficiency at the site of disease is attributable to the same, different or interacting factors that regulate systemic levels. Since soluble RAGE has multiple protease sensitive sites (Kumano-Kuramochi *et al.*, 2009), the marked deficiency at the site of disease might be augmented by increased degradation by proteases from infiltrating cells. Supporting this idea, we demonstrated that recovery of exogenously added soluble RAGE was approximately 45% less in bronchial lavage samples from neutrophilic versus non-neutrophilic subjects with asthma or COPD (Sukkar *et al.*, 2011).

While loss through degradation is one hypothesis that requires further investigation, other mechanisms might also contribute. In this regard, it is important to remember that soluble RAGE is produced by ectodomain cleavage and shedding or alternative mRNA splicing and secretion, and thus distinct mechanisms related to these separate processes might be responsible. Currently, there are no tools to determine readily the extent to which cleaved versus secreted forms of soluble RAGE contribute to the extracellular pool of total soluble RAGE (Yamamoto *et al.*, 2007). However, we observed a strong correlation between concentrations of total soluble RAGE and the secreted form (RAGE_v1) in the bronchial lavage fluid and serum, in healthy subjects and in asthmatic and COPD subjects, irrespective of the presence or absence of airway neutrophilia. Thus, RAGE_v1 might be the dominant form of circulating soluble RAGE in humans, but further clarification of this issue is needed.

Does soluble RAGE deficiency promote neutrophilic inflammation in chronic airways disease?

In addition to regulating neutrophil trafficking to sites of inflammation (Chavakis *et al.*, 2003; Orlova *et al.*, 2007; Zen *et al.*, 2007; van Zoelen *et al.*, 2009; Frommhold *et al.*, 2010), the ligand–RAGE axis also contributes to the resolution of inflammatory response. Indeed, two independent studies have shown that phagocytosis of apoptotic cells by macrophages is mediated by RAGE-dependent recognition of phosphatidylserine, an 'eat me' signal expressed on apoptotic cells (Friggeri *et al.*, 2011; He *et al.*, 2011). Intriguingly, in an LPS model of acute lung injury macrophage phagocytic activity and apoptotic neutrophil clearance was impaired in RAGE-deficient mice (He *et al.*, 2011). Furthermore, recombinant soluble RAGE impaired macrophage phagocytic activity, indicative that soluble RAGE competes with membrane RAGE for binding to phosphatidylserine, and thus inhibits neutrophil clearance. Thus, on the basis of this evidence, deficiency in lung soluble RAGE would favour the clearance of neutrophils, rather than promote their accumulation.

So the question arises as to why deficient levels of lung soluble RAGE are associated with airway neutrophilia in asthma and COPD? In subjects with cystic fibrosis, airway neutrophilia is persistent and their airway neutrophils, compared with circulating neutrophils, exhibit increased RAGE expression and activation of signalling molecules downstream of RAGE ligation (CREB transcriptional co-activator). Moreover, these phenotypic changes are associated with 100-fold higher levels of the RAGE ligand S100A12 in the lung versus the systemic compartment, and undetectable levels of lung soluble RAGE (Makam *et al.*, 2009). Thus, deficiency in lung soluble RAGE appears to allow for unopposed and sustained RAGE-dependent neutrophil entry into the lung.

Interestingly, Watanabe *et al.* (2011) observed increased sputum levels of HMGB1, together with increased (rather than decreased) sputum levels of the secreted form of soluble RAGE (RAGE_v1) in asthmatic subjects. A significant correlation between sputum levels of HMGB1 with disease severity, lung function impairment and the number of sputum neutrophils was also observed; while there was no correlation between sputum levels of soluble RAGE and neutrophils. Of note, however, the subjects studied by Watanabe and col-

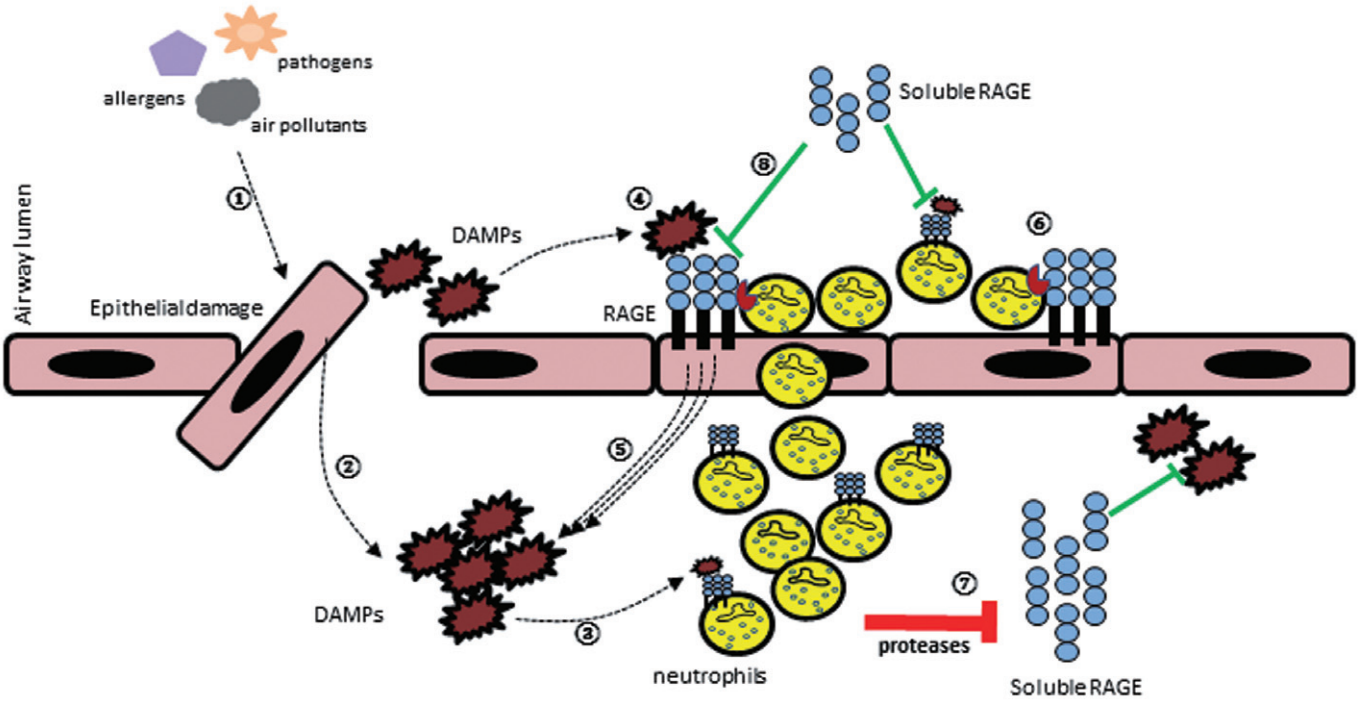

Figure 2

Proposed schematic of the relationship between soluble RAGE and neutrophilic inflammation in chronic airways disease. Epithelial damage (1) caused by environmental insults induces the release of DAMPs (e.g. the RAGE ligands HMGB1, SAA) (2). DAMPs induce recruitment and activation of neutrophils in the airway space (3). DAMPs might also act in an autocrine manner to activate RAGE on airway epithelial cells (4), leading to the generation of further DAMPs (5), thus amplifying the inflammatory response. Activated neutrophils up-regulate the RAGE ligand Mac-1/CD11b on their surface and might therefore interact with airway epithelial cells via a RAGE-dependent mechanism (6). Neutrophil-derived proteases degrade soluble RAGE (7), the endogenous inhibitor of RAGE signalling (8), leading to unopposed neutrophil recruitment, activation and persistence in the airways and a failure to resolve the inflammatory response.

leagues had lower levels of airway neutrophils (median 24%, upper range 47%) compared with neutrophilic asthmatic and COPD subjects that we studied, where levels of airway neutrophils were greater than 65%, and soluble RAGE was profoundly deficient (Sukkar *et al.*, 2011). Thus, it might be possible that expression of lung soluble RAGE is increased to counteract ligand-RAGE induced neutrophilia; but if a tipping point is reached where the increased secretion of soluble RAGE is insufficient to counteract proteolytic degradation by excess levels of neutrophil derived enzymes, the neutrophil response will ensue (Figure 2).

Clearly, further work is needed to untangle what appears to be a complex relationship between lung soluble RAGE and neutrophilic inflammation in chronic airways disease. However, at least for now, it seems that neutrophils might contribute to disease pathogenesis by eliminating protective factors such as soluble RAGE, and that correcting deficiencies in lung soluble RAGE might provide the critical brake needed to arrest neutrophilic inflammation; a hypothesis that warrants testing in future studies.

Impact of environmental pollutants and cigarette smoke on lung RAGE expression

While Ferhani *et al.* (2010) demonstrated increased RAGE and HMGB1 expression in the airways of smokers with COPD, compared with smokers without COPD, the latter group still had significantly greater levels of RAGE and HMGB1 expression in airway mucosal cells compared with never smokers. This suggests that the ligand–RAGE axis might be activated as an early response to environmental insults to protect lung integrity, but that in genetically susceptible individuals, this may become a pathogenic response. Reports that lung RAGE protein expression is increased following chronic smoke exposure in emphysema-susceptible but not emphysema-resistant strains of mice provides some support for this concept (Reynolds *et al.*, 2008).

Studies in animal models demonstrate the capacity for cigarette smoke, and other environmental pollutants, including particulate matter, and pollutant gases to increase lung RAGE expression (Xu *et al.*, 2008; Zhang *et al.*, 2008c; 2009; Kodavanti *et al.*, 2011). Using a proteomics approach, Zhang *et al.* (2008c; 2009) demonstrated increased lung RAGE expression in rats exposed to cigarette smoke for prolonged periods. In contrast, increased lung RAGE mRNA expression was observed in rats subjected to acute but not chronic exposures of diesel exhaust particles (DEPs) (Kodavanti *et al.*, 2011). Interestingly, while chronic exposure to ozone and/or DEPs had no effect on RAGE expression in heart or lung tissue, acute combined exposure to ozone and DEPs significantly increased RAGE and HMGB1 expression in aortic tissue. The lack of effect of ozone exposure on lung RAGE expression in this study might have been due to the low

concentration of ozone (0.5 ppm) used. Ozone at higher concentrations (2–3 ppm) induces airway hyperresponsiveness and airway inflammation via TLR2- and TLR4-dependent pathways in experimental models of asthma (Williams *et al.*, 2007; Peden, 2011). Thus, it will be important to determine whether RAGE is involved in the airway response to ozone and whether it augments the response through interaction with TLR4 signalling.

Functional consequences of environmental pollutants and cigarette smoke on RAGE expression and function in alveolar epithelial cells

Type I alveolar epithelial (AT1) cells are large squamous cells whose thin cytoplasmic extensions form the air–blood barrier required for normal gas exchange. These cells cover ~98% of the internal lung surface area, and while previously thought to play a passive role in forming the air–blood barrier, it is now recognized that they have broad physiological roles important for alveolar function (Dobbs *et al.*, 2010). As mentioned above, RAGE is a differentiation factor for AT1 cells (Dahlin *et al.*, 2004; Shirasawa *et al.*, 2004; Demling *et al.*, 2006; Reynolds *et al.*, 2008). It is localized to the luminal edges and basal membrane of these cells and has been shown to promote their adherence and spreading (Fehrenbach *et al.*, 1998; Demling *et al.*, 2006; Reynolds *et al.*, 2008). Of interest, transgenic mice that over-express RAGE exhibit profound abnormalities in alveolar epithelial cell differentiation that culminate in severe respiratory distress and perinatal lethality, highlighting the importance of RAGE for normal alveolar function (Reynolds *et al.*, 2011c).

Recent studies by Reynolds *et al.* (2008; 2011a) have shown that environmental pollutants activate AT1 transcriptional programmes that involve amplification of RAGE expression and signalling. Cigarette smoke extract and diesel particulate matter increase RAGE expression in R3/1 cells, an AT1-like cell line. Moreover, RAGE is a transcriptional target of egr-1, a transcription factor that is itself induced in response to cigarette smoke extract or following activation with AGEs in R3/1 cells (Reynolds *et al.*, 2008). Thus, the authors suggest that a positive feedback cycle mediated by egr-1 might contribute to persistent up-regulation of alveolar epithelial RAGE expression and activation in smoking-related airways disease. In further studies, they demonstrate that cigarette smoke-induced Ras and NF-κB activation occurs down-stream of RAGE ligation in R3/1 cells and A549 cells, an ATII-like cell line. These findings were corroborated by evidence of reduced Ras activation, and NF-κB-dependent inflammatory gene expression in the lungs following chronic smoke exposure in RAGE-deficient mice compared with their wild-type counterparts (Reynolds *et al.*, 2011b). Similarly, RAGE-dependent NF-κB activation and inflammatory gene expression was observed in R3/1 cells exposed to diesel particulate matter (Reynolds *et al.*, 2011a). Since RAGE is known to be a transcriptional target of NF-κB (Tanaka *et al.*, 2000), this is potentially another pathway by which cigarette smoke exposure might lead to the amplification of RAGE expression and signalling in AT1 cells.

The observation that cigarette smoke activates down-stream transcriptional programmes via RAGE ligation raises the question of whether this response is due to AGEs formed by reactive glycation products present in cigarette smoke (Cerami *et al.*, 1997), or is due to smoke-induced release of endogenous RAGE ligands such as HMGB1 (Reynolds *et al.*, 2008; Bezerra *et al.*, 2011). It is more than likely that both mechanisms contribute to the availability of ligands, with further studies needed to determine the identity of the specific ligands involved.

Does RAGE play a role in the activation of innate and adaptive immune responses in chronic airways disease?

In addition to initiating the innate inflammatory response and immediate host defence, the activation of innate immune receptors is proposed to shape the type of adaptive immune response that is generated. Yet despite the overwhelming evidence that the RAGE axis is altered in chronic airways disease, the role of RAGE signalling in programming appropriate and inappropriate adaptive immunity remains unresolved. No doubt this question will be addressed in the coming years; but several clues, as discussed below, suggest it is almost certain to emerge as a key player.

Potential for RAGE-TLR4 collaboration

The first clue is the emerging notion that RAGE co-operates with members of the TLR family to facilitate the recognition of ligand complexes involved in the amplification of immune and inflammatory responses, as discussed earlier. The possibility of RAGE/TLR4 co-operation in the context of airways disease is of particular interest, as TLR4 is a key pattern-recognition receptor implicated in the airway response to various environmental stimuli. Indeed, both we and others have shown that house-dust mite (HDM)-induced allergic inflammation and AHR is mediated through ligation of TLR4 and MyD88 signalling at the airway mucosal surface (Hammad *et al.*, 2009; Phipps *et al.*, 2009). The capacity for HDM to engage TLR4 signalling is believed to occur through functional mimicry between the HDM allergen Der p2 and MD-2: the LPS-binding component of the TLR4 signalling complex (Trompette *et al.*, 2009). TLR4 ligation on airway epithelial cells induces the release of innate cytokines such as IL-25, IL-33 and TSLP which promote the development of pathogenic $CD4^+$ T helper 2 (Th2) cells and asthmatic inflammation (Hammad *et al.*, 2009). On the other hand, TLR4 has also been implicated in the airway response to ozone in animal models (Hollingsworth *et al.*, 2004; Williams *et al.*, 2007). In this case, low molecular weight hyaluronan, which is an endogenous ligand for TLR4, is generated upon ozone-induced airway injury and is believed to be the factor responsible for ozone-induced AHR (Garantziotis *et al.*, 2009a,b). Finally, TLR4-deficient mice are protected against pulmonary inflammation following acute cigarette-smoke inhalation in mouse models (Doz *et al.*, 2008). Together, these studies demonstrate how diverse environmental stimuli converge on TLR4 signalling, either via direct receptor interactions, or through the secondary release of endogenous damage-associated molecular patterns, in experimental models of asthma and COPD. Yet whether RAGE facilitates or enhances

ligation of TLR4 in response to these stimuli remains an open question and is therefore an important area of future investigation, particularly given evidence of RAGE expression and activation in response to environmental pollutants and particulates, as discussed above.

RAGE regulates T-helper cell differentiation and polarization

The second clue is the emerging position of the ligand–RAGE axis as a determinant of adaptive immune activation and particularly the functional activation and polarization of T helper responses. Through the expression of multiple pattern-recognition receptors, dendritic cells lining the airway mucosal surface are endowed with the ability to detect pathogen- or damage-associated molecular patterns. Ligation of pattern-recognition receptors in dendritic cells induces their maturation and migration to the draining regional lymph nodes where they instruct the activation and differentiation of antigen-specific T cells. Thus, dendritic cells are seen as important determinants of the host response to the external environment and a critical nexus bridging the innate and adaptive immune response. Evidence from *in vivo* studies suggests a role for RAGE in the mobilisation of activated dendritic cells from peripheral tissue sites to regional lymph node areas (Manfredi *et al.*, 2008). Moreover, evidence from *in vitro* studies suggests that ligand–RAGE interactions are involved in dendritic cell–T-cell cross-talk, playing a role in the clonal expansion, survival and functional polarization of $CD4^+$ T cells. When immature (or resting) dendritic cells are stimulated with pathogen-associated molecular patterns, such as LPS or CpG DNA, they release HMGB1 that signals via RAGE in an autocrine fashion to induce their maturation and migration; thus, the HMGB1–RAGE pathway has been implicated as an autocrine loop driving dendritic cell maturation and mobilization (Messmer *et al.*, 2004; Dumitriu *et al.*, 2005a,b; 2007; Yang *et al.*, 2007a; Campana *et al.*, 2009; Zhu *et al.*, 2009).

Although early studies indicated that RAGE ligation favours a Th1 bias, it is becoming increasingly apparent that, depending on the type of ligand and other yet to be identified signals, it may also promote the development of Th2 and Th17 responses. Inhibition of HMGB1 or RAGE activity during dendritic cell activation with the TLR4 ligand LPS decreased the numbers of IFN-γ-positive $CD4^+$ T cells. This effect was associated with reduced dendritic cell secretion of the Th1 polarizing cytokine IL-12 p70 (Dumitriu *et al.*, 2005b). In further studies, Moser *et al.* (2007) showed that RAGE-deficient $CD4^+$ T cells exhibit impaired proliferative responses and reduced IFN-γ production, indicating that ligation of RAGE on T cells is important for their differentiation towards a Th1 phenotype. Moreover, RAGE-deficient $CD4^+$ T cells stimulated with CD3/CD28 appear to release greater amounts of the Th2 cytokines IL-4 and IL-5, suggesting that RAGE might negatively regulate Th2 functional responses (Chen *et al.*, 2008).

In contrast to the above, recent studies suggest that under certain conditions RAGE ligation promotes expansion of Th2 type cells which secrete IL-4, IL-5 and IL-13. Interestingly, Buttari *et al.* (2011) showed that when co-cultured with allogenic $CD4^+$ T cells, AGE-modified β2 glycoprotein I-stimulated dendritic cells induce greater numbers of IL-4 producing T cells, compared with IFN-γ-producing T cells, indicating that AGE–RAGE interactions can favour a Th2 rather than a Th1 bias. Consistent with this, Hilmenyuk *et al.* (2010) observed greater production of IL-5, and reduced production of IFN-γ, in $CD4^+$ T cells co-cultured with dendritic cells pulsed with AGE-modified OVA compared with those pulsed with regular OVA. Of note, when AGE-BSA pulsed dendritic cells were co-cultured with allogenic $CD4^+$ T cells, significant production of IL-2 is observed (Ge *et al.*, 2005), which is of interest because IL-2 is an important determinant of T-helper cell differentiation, influencing the expansion of multiple T-cell lineages, including Th1, Th2 and Th17 subsets (Liao *et al.*, 2011). It will be important to determine whether ligation of RAGE as a result of distinct environmental exposures drives the polarization of Th2/Th17 versus Th1/Th17 responses implicated in sustaining the inflammatory response in asthma and COPD respectively.

RAGE ligands polarize T-helper responses in the lung

The final clue is the emerging list of endogenous RAGE binding ligands, such as SAA, C3a and HMGB1 implicated in the immunopathology of chronic airways disease. While SAA proteins are primarily expressed by hepatocytes during the acute phase response, extra-hepatic expression is observed under normal physiological conditions in the epithelial compartment of most organ tissues, including the lung (Urieli-Shoval *et al.*, 1998; Upragarin *et al.*, 2005; Chen *et al.*, 2010a; Sung *et al.*, 2010). Indeed, increased expression of SAA is observed in the lung tissue across diverse pathological conditions, including asthma and COPD (Ozseker *et al.*, 2006; Chen *et al.*, 2010a; Sung *et al.*, 2010; Bozinovski *et al.*, 2012). Furthermore, local expression of SAA is inducible in the lungs of mice subjected to various stimuli that induce experimental asthma or COPD, including those employing acute sensitisation/challenge protocols with ovalbumin/aluminium hydroxide or acute exposures to LPS, NO_2 or cigarette smoke (Jaradat *et al.*, 2006; Ray *et al.*, 2006; Ather *et al.*, 2011; Bozinovski *et al.*, 2012). Of note, local delivery of SAA into the airways in mice induces neutrophil infiltration into the lung, along with increased expression of pro-neutrophilic cytokines and chemokines (Ather *et al.*, 2011; Bozinovski *et al.*, 2012). Furthermore, *in vitro* stimulation of bone-marrow-derived dendritic cells with SAA favours the development of Th17 responses and associated production of IL-17, which is linked to the development of neutrophilic inflammation in asthma and COPD (Brusselle *et al.*, 2011; Wang and Wills-Karp, 2011). Finally, sensitisation of mice with OVA in the presence of alum was shown to elicit a Th2 response, as expected, however, when SAA was used as the adjuvant the mice produced a Th17 response. Of note, another endogenous RAGE binding ligand C3a was also found to mediate severe allergen-induced airway hyperresponsiveness driven by Th17-dependent inflammation (Lajoie *et al.*, 2010; Ruan *et al.*, 2010). Further studies are needed to determine whether RAGE ligation is involved in the airway response to SAA and C3a, and furthermore, whether other RAGE ligands such as HMGB1, which is also released into the airway space in response to allergen or smoke exposures in animal models (Hou *et al.*, 2010; Bezerra *et al.*, 2011), contribute to airway immunopathology in chronic airways disease.

Conclusions and future directions

Asthma and COPD are complex heterogeneous disorders of the respiratory tract that have significant genetic and environmental components. The underlying immunopathology, although distinctly different between the two conditions, arises from the activation of innate immune pathways by various environmental exposures such as oxidizing pollutants, particulate matter, bioactive allergens and respiratory pathogens in genetically susceptible individuals. There is now intense research activity directed at identifying the pattern-recognition receptors involved in responding to these environmental signals, and the associated innate and adaptive immune responses that lead to perpetuation of the inflammatory response. Recent studies demonstrate heightened expression of RAGE and its ligands in chronic airways disease, together with reduced expression of soluble RAGE, the endogenous inhibitor of RAGE signalling. Since RAGE is capable of interacting with a large repertoire of endogenous molecules released by stressed, injured or inflamed tissues, it needs to be determined whether dysregulation of ligand-RAGE signalling contributes to immunopathology in asthma and COPD.

Evidence that cigarette smoke and environmental pollutants induce lung RAGE expression, and engage RAGE signalling in airway cells provides a compelling basis for RAGE in COPD pathogenesis. Of note, RAGE ligation activates oxidant pathways that lead to generation of reactive oxygen species; thus whether RAGE signalling increases lung oxidant burden, a key pathogenic factor in COPD, is an important area of future research. There is evidence of increased expression of RAGE ligands in asthmatic subjects, while RAGE ligands such as SAA and C3a are induced following allergen challenge in experimental asthma. Indeed, since SAA and C3a are associated with the development of Th17 responses and neutrophilic inflammation, key features of more severe types of asthma, this raises the intriguing question of whether ligand–RAGE signalling is a molecular determinant of certain phenotypes, or endotypes of asthma. Our studies demonstrating marked deficiency in lung soluble RAGE in neutrophilic phenotypes of asthma and COPD further support this concept and suggest it might also extend to COPD. It is important to now determine whether this apparent deficiency is a benign consequence of proteolytic degradation attributed to excessive levels of neutrophil-derived proteases, or whether it allows for continued and unopposed neutrophil recruitment into the lung.

Ligation of TLRs by environmental pollutants and allergens is recognised as an important step towards the activation of innate and adaptive immune responses, and airway pathology in asthma and COPD. It is now important to determine whether co-ligation of RAGE, and collaborative RAGE/TLR signalling provides another level of discrimination and regulation, which dictates the shape and nature of the ensuing immune/inflammatory response, and in particular the differential polarisation of T helper responses towards Th1, Th2 or Th17. Moreover, while the role of RAGE in respiratory infection was outside the scope of this review, given RAGE is capable of directly binding pathogen-derived products, and also appears to collaborate with TLRs in the recognition of pathogen-derived ligands, it will be important to determine whether RAGE is implicated asthma and COPD exacerbation, which is frequently associated with respiratory infection.

Finally, while RAGE gene polymorphisms appear to be related to spirometric measures of airflow obstruction in healthy individuals, further work is needed to establish the relationship between RAGE gene polymorphisms, environmental exposures and susceptibility to asthma and COPD across different populations.

Therapeutic implications

Inhibitors of RAGE signalling are currently in clinical development for Alzheimer's disease; thus, if future studies identify a significant pathogenic role for ligand–RAGE signalling in asthma and COPD, trial of these agents in the context of airways disease might lead to novel therapeutics for the management of these conditions in the not too distant future. Interestingly, a phase II clinical trial (NCT00566397) of a small molecule RAGE inhibitor (PF-04494700/TTP488) in Alzheimer's patients was recently discontinued due to poor efficacy. Nevertheless, another large molecule RAGE inhibitor (TTP4000), which is a fusion between the ligand binding domains of RAGE and IgG, is still under clinical development and is entering a clinical study in Alzheimer's patients (Transtech Pharma Inc).

Further to inhibitors in clinical development, several strategies including RAGE-IgG fusion proteins (Li *et al.*, 2011a), humanized anti-RAGE monoclonal antibodies (Vugmeyster *et al.*, 2010; Christaki *et al.*, 2011), recombinant soluble RAGE (Park *et al.*, 1998; Zhang *et al.*, 2008a; Yamamoto *et al.*, 2011) and low molecular weight heparin (Myint *et al.*, 2006; Rao *et al.*, 2010) have been employed to inhibit RAGE signalling in pre-clinical models across diverse pathologies. Targeted inhibition of RAGE signalling has so far focused on inhibiting the ligand–RAGE interaction without any consideration for ligand specificity. Given the RAGE axis is central to the host immune response, strategies that target specific ligands that underlie disease pathogenesis in asthma and COPD will be more desirable than those that generically block receptor function. Such strategies might be possible with further characterisation of the mechanisms by which different ligands engage the receptor (Fritz, 2011).

Finally, it is important to mention that certain therapeutic drug classes, including statins, thiazolidinediones, ACE inhibitors and angiotensin II type 1 receptor antagonists, have been shown to either increase serum levels of soluble RAGE, or decrease expression of the membrane receptor (Lanati *et al.*, 2010). As these drug classes are thought to have therapeutic potential in airways disease (Barnes, 2008a; Belvisi and Mitchell, 2009; Young *et al.*, 2009; 2011), it will be important to determine whether their therapeutic benefit is due to activity at the level of the RAGE axis and, further to that, how they might be used to target the RAGE axis in airways disease.

Conflicts of interest

None.

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